DELHI PHARMACEUTICAL SCIENCES & RESEARCH UNIVERSITY

(Incorporating DIPSAR as Constituent College as per DPSRU Act)

Synopsis

for

Doctor of Philosophy (Ph.D)

Govt. of N.C.T. of Delhi,

Pushp Vihar, Sector – III, M.B. Road,

New Delhi-110017

Website: www.dpsru.edu.in

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Accreditation by Life Sciences Sector Skill Development Council

DELHI PHARMACEUTICAL SCIENCES & RESEARCH UNIVERSITY

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Registration No.: 31/PhD/DPSRU/2018

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PhD Supervisor: Prof. Pravat K. Sahoo

Project Title: Development and evaluation of intranasal drug delivery of Dopamine hydrochloride loaded Nanostructured Lipid Carrier for effective treatment of Parkinson's disease.

Project Synopsis

Introduction

For the treatment of CNS illnesses, numerous neurotherapeutic have failed to successfully deliver the medicine to the brain. Parkinson's disease is the second most prevalent neurodegenerative diseases of the central nervous system, characterized by movement abnormalities and cognitive deficiencies. Over 1% to 2% of the general population over the age of 60 is impacted, and approximately 5 million people worldwide are afflicted. Due to the physiological barriers of the brain known as the blood brain barrier (BBB) and blood cerebrospinal fluid barrier (BCSFB), CNS medication delivery is a challenging feat. The BBB prevents the oral and intravenous transfer of medications to the brain, and as a result, a number of treatments are ineffective in treating CNS illnesses. The primary goal of Parkinson's disease treatment is to increase the normal concentration of dopamine in the brain, but due to its lipophobic nature, dopamine cannot pass the blood-brain barrier. To improve the characteristics of dopamine, an intranasal formulation of nanostructured lipid carrier (NLC-DOPA) was used to bypass the blood-brain barrier and eliminate the problem of Levodopa and Ritalin treatment. NLC of dopamine is a novel method of medication administration that does not modify the chemical molecule. To prepare NLC-DOPA with solid liquid lipid, surfactant, and cosurfactant as the primary excipients, high-temperature, high-pressure homogenization and various assessments are chosen. Consequently, it is anticipated that intranasal administration of NLC-DOPA will be superior to invasive methods of drug delivery to the brain in terms of enhanced drug absorption and reduced systemic side effects.

Rationale

1) The fundamental goal of Parkinson's disease therapy is to increase the normal concentration of dopamine in the brain, yet dopamine does not cross the BBB due to its hydrophilic nature.

2) Levodopa is a prodrug of dopamine can cross the BBB and convert into dopamine by the naturally occurring enzyme DOPA decarboxylase but it has bioavailability problem (30%) and very low brain uptake due to its metabolization by aromatic amino acid decarboxylase and catechol-o- transferase in the peripheral circulation before crossing BBB. Hence, Levodopa is co-administered with carbidopa which neither cross BBB nor produce

pharmacological action but inhibit degradation of Levodopa in peripheral circulation by inhibiting amino acid decarboxylase. But this combination has some simultaneously side effects with long term treatment.

Objective

To develop and evaluate dopamine (DOPA) hydrochloride loaded NLC drug delivery system to bypass the blood brain barrier using intranasal route of administration with improve compliance, bioavailability and less side effect for Parkinson's disease.

Methodology

- Preparation and optimization of dopamine loaded nano structure lipid carrier was performed using drug (dopamine hydrochloride) and other excipients (solid lipid-cholesterol, lipids, surfactant and co surfactant) via a homogenization at 20000 rpm for 20min and ultra probe technique at 70^0 C, 60% AMP for 15min that was optimized by design expert 13 software.
- For the analysis of NLC-DOPA formulation the method of validation was developed by UV spectroscopy and HPLC techniques to find out the linearity, precision, system suitability, specificity, accuracy, assay and robustness.
- The confirmatory optimization was achieved by in-vitro release kinetic of different formulations with the help of DDS software and characterized by zeta-sizer, TEM, SEM, XRD, FTIR analysis.
- The pharmacokinetic study was performed by liquid- liquid extraction method using HPLC, the parameters were calculated by PK Solver software.
- The pharmacodynamic study was performed by rotenone induced model and the extant of drug from NLC-DOPA was estimated by ELISA test.
- The ex-vivo studies such as permeation of drug and ciliotoxicity effect during administration were performed using Franz-diffusion method and exhibited by histopathology images.
- According to ICH guideline stability for optimized formulation was performed for six months.

Result and Discussion

Optimization

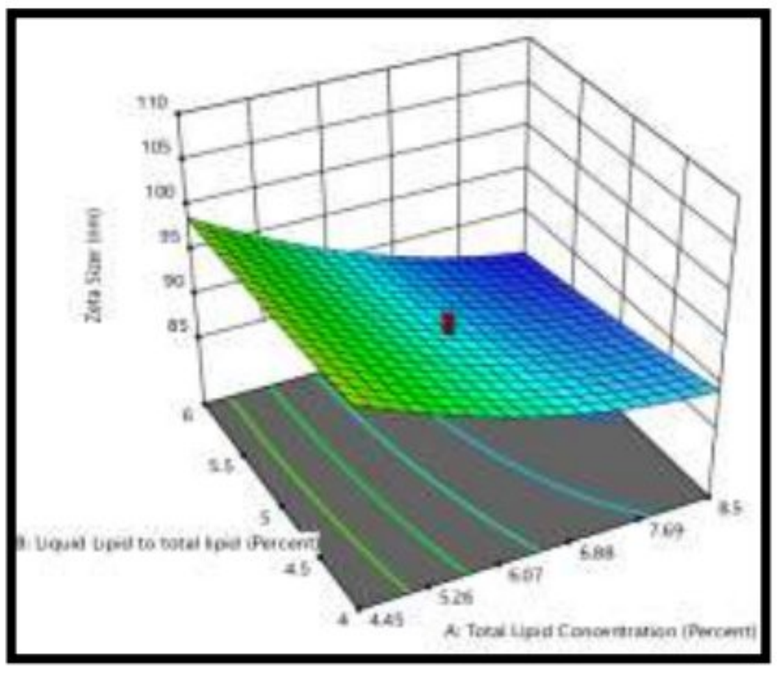
Table 1. Design Composition of NLC- DOPA formulation

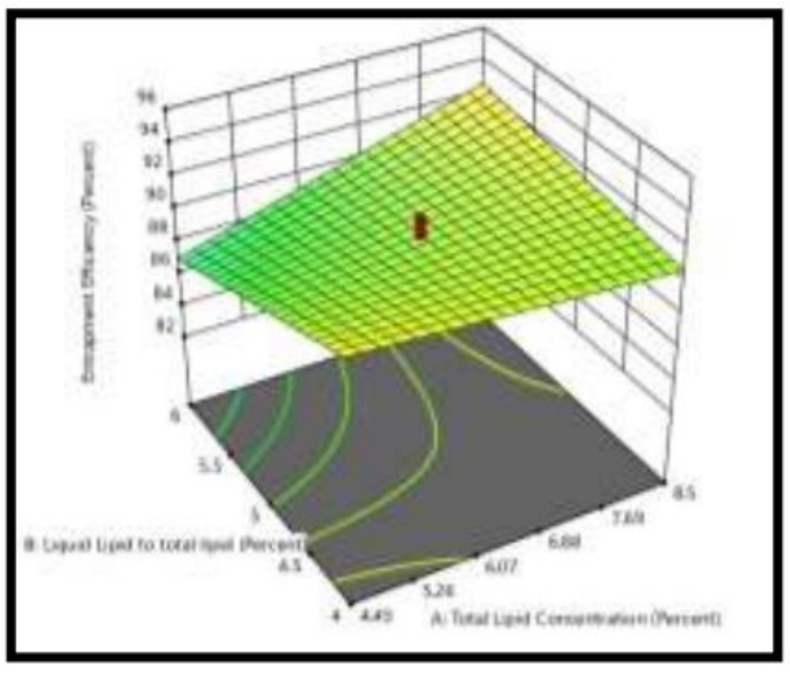
S.No.	Ingredients	Quantity (%)	
		Low	High
1	Total lipid concentration	4.45	8.5
2	Liquid lipid to total lipid concentration	4	6
3	Surfactant concentration	2.5	5
4	Drug Concentration	0.05	
5	Co surfactant	2.5	
6	Millipore water	q.s	

The central composite design was implemented to systematically assess the effect of structural parameters affecting the physico-chemical properties of NLC-DOPA. It was well suited for a quadratic surface, which generally performed well with the fine-tuning process.

A total 20 trials were conducted to find out the impact of structural variability on particle size (PS) and entrapment efficiency (EE). Response rates Y1 (PS) and Y2 (EE) ranged from 85.53 to 106.11nm and from 82.17 to 95.47%, respectively.

The 3D plots have confirmed that particle size considerably enhanced with rising total lipid concentration and reduced with enhanced liquid lipid to entire lipid concentration. The 3D graph of entrapment efficiency enhanced considerably with rising entire lipid concentration and reduced with liquid lipid to entire lipid concentration.





(a) **(b)**

Fig.1 3-Dimentional (3-D) response surface graph of response Y1(a) and Y2(b)

Validation

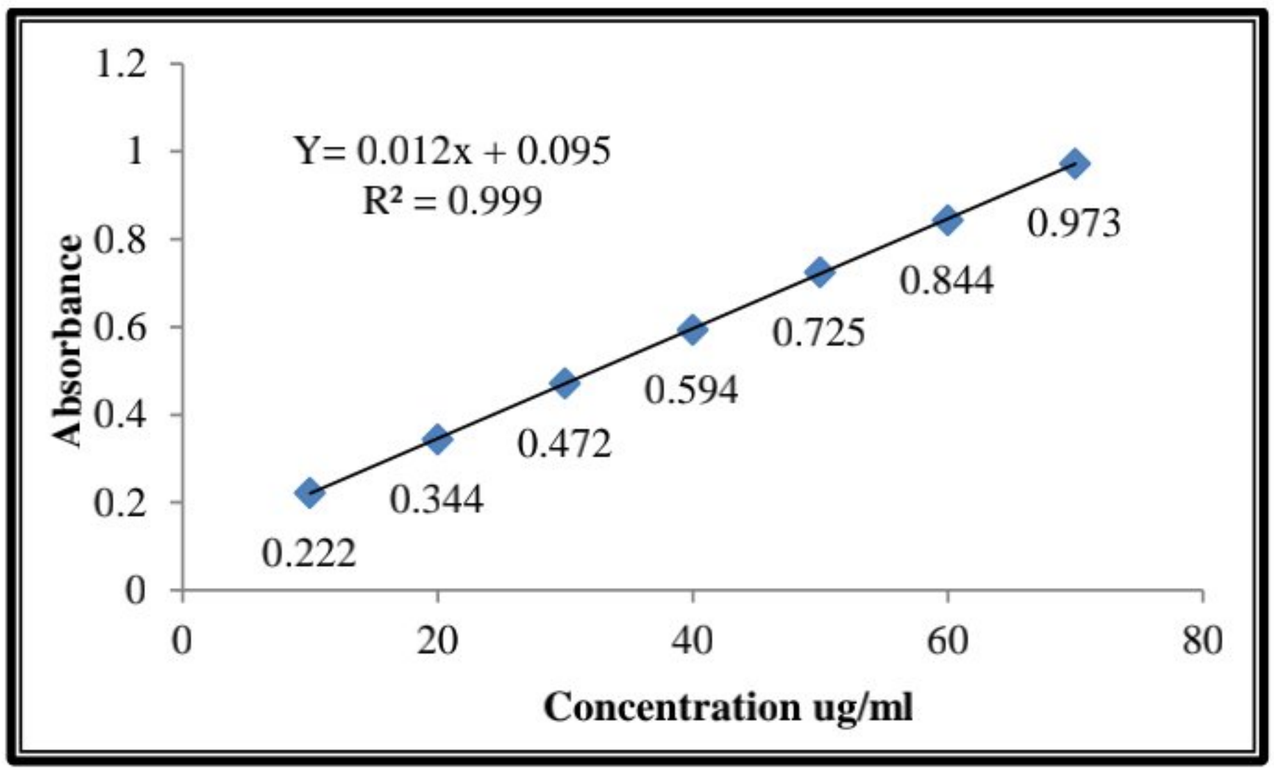


Fig. 2 Calibration plot of dopamine hydrochloride in 6.4 pH sodium phosphate buffer at 280nm $\lambda_{max.}$

Table 2. Method validation parameters for NLC-DOPA formulation by UV method

Sr. No.	Validation Parameters	Remark
1.	Linear Equation	y = 0.012x + 0.095
	R^2	0.999
	Range	10-70 µg/ml
2.	Precision	%RSD
	Intra day	0.86%-1.91%
	Inter day	1.33%-1.59%
3.	Limit of Detection (LOD)	0.56 µg/ml
4.	Limit of Quantification (LOQ)	1.7 µg/ml
5.	Assay (Mean ±%RSD)	99.7%±0.47%
6.	Accuracy	Mean ±%RSD
	40%	100.8 ±0.9

	80%	99.37±0.96
	120%	100.98±1.21
7.	Robustness	Robust
8.	Specificity	Specific

Table3. Method validation parameters for NLC- DOPA formulation by HPLC technique

Validation Parameters		**Remark**
Mobile phase:		0.05 M KH_2PO_4 (pH 2.5) and Methanol (90:10)
System Suitability parameters	Peak impurity	Not detected
	Peak Purity index	1.000000
	The number of theoretical plates	7716.788
	Tailing Factor	1.230
	Peak area (Mean ± %RSD)	1170357 ± 0.43
	Retention time (Mean ± %RSD)	4.872 min
Linearity	Equation	Y = 15074X - 15743
	R^2	0.999
	Range	20 to 100 µg/ ml
Precision	Intra day	0.28- 1.1%
	Inter day	0.25-0.56%
Limit of Detection (LOD)		3.7 µg/ ml
Limit of Quantification (LOQ)		11.2 µg/ml
Selectivity/specificity		Specific and Clear
Accuracy	40%	99.43% ± 0.2
	80%	99.58% ± 0.28

	120%	99.53% ± 0.27
Assay (Mean ±%RSD)		99.21% ±0.001
Robustness		Robust

In vitro Evaluation

Drug Kinetic Release

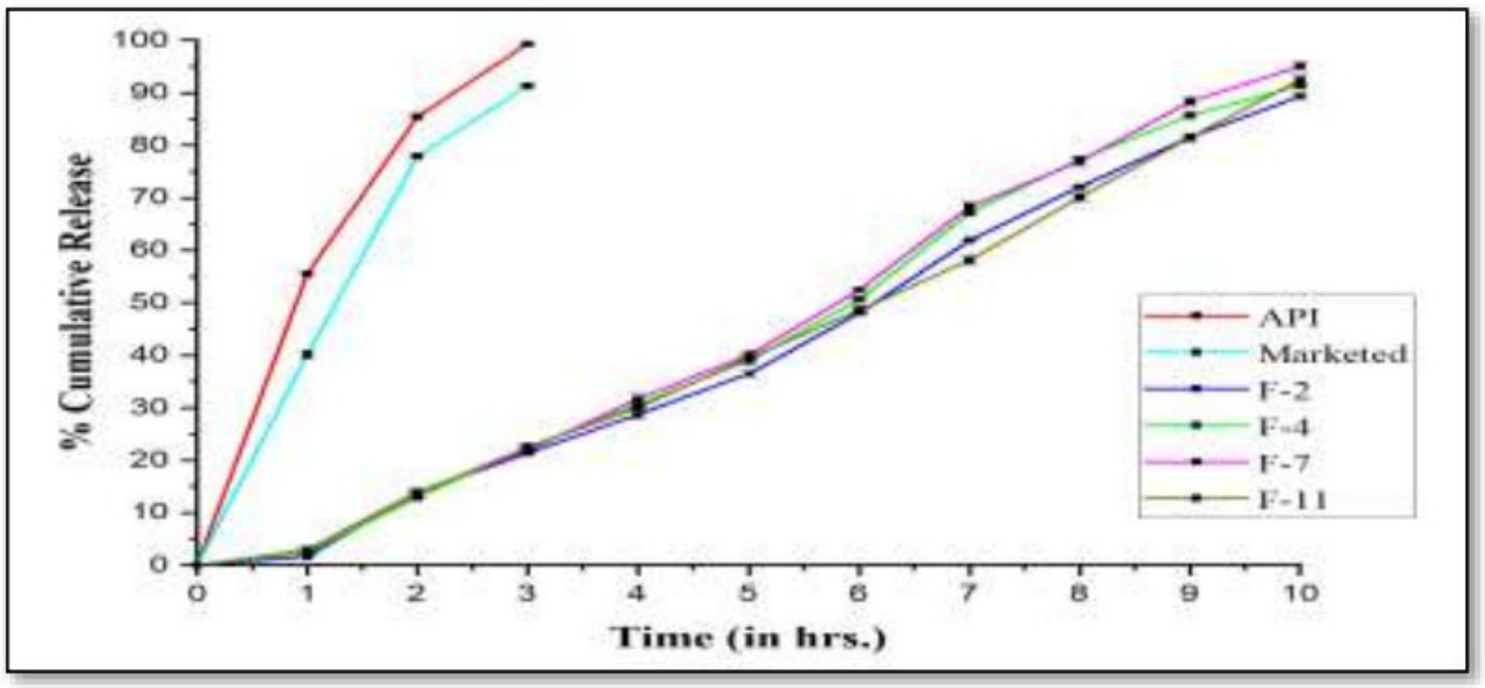


Fig.2 In vitro drug release profile of API, Marketed Levodopa (M) and NLC-DOPA formulations F2, F4, F7, F11

DDSolver software was used to find out kinetic release of drug. The release data of drug from NLC-DOPA formulations were fitted to various kinetic models, including zero order, first order, Higuchi's equation, Hixon Crowell's equation, and Korsmeyer-Peppas' equation, to determine the best-fitted model and the mechanism of drug kinetic release.

On the basis of DDSolver, the best fitted release kinetic model of optimized formulations (F2, F4, F7, and F11) exhibited a predetermined sustained and controlled release, followed Krosemeyer Peppas's equation and zero order kinetic release, and had good R2 adjusted, Akaike information criterion (AIC), and Model selection criterion (MSC) when compared with API and marketed Levodopa release.

Table 4. Best fitted kinetic models for NLC-DOPA formulation

Kinetic Models	Goodness of Fit (Optimized formulation F11)			
	K	Rsqr_adj	AIC	MSC
Zero Order	8.657	0.9797	52.7679	3.6996

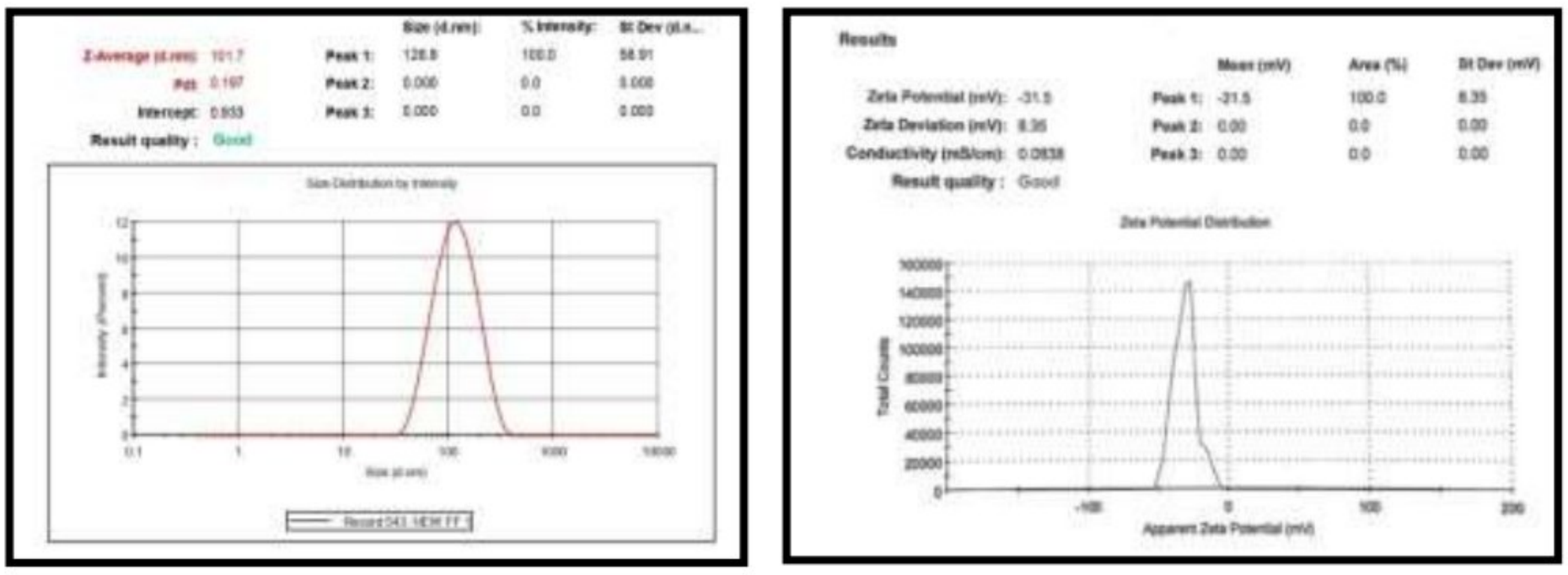
First Order	0.129	0.8664	71.6333	1.8131
Higuchi Model	22.138	0.7399	78.2963	1.1467
Hixson-crowell	0.038	0.9072	67.9892	2.1775
Korsmeyer-peppas Model	5.412 n=1.232	0.9983	29.0728	6.0691

Entrapment efficiency % and Drug loading %

The entrapment efficiency of NLC-DOPA was found to be from 82.17to 95.47 % and the drug loading of final formulation was found to be 23.246%.

Characterization

Zeta sizer and Zeta potential of optimized NLC-DOPA formulation

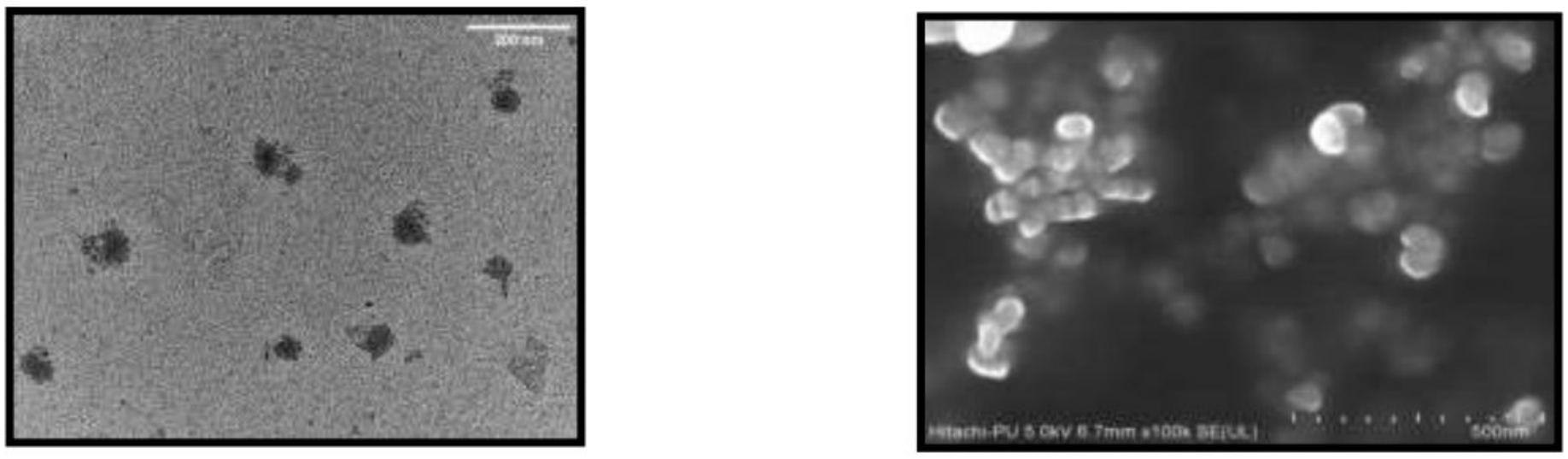


(a) **(b)**

Fig. 3 Zeta sizer (a) and zeta potential (b) graph

The optimization of NLC-DOPA formulation based on particle size (nm) range from 85.53 to 106.11nm, the value of zeta sizer of optimized formulation was found to be 90.38nm. The surface charge property (positive or negative) of particles was determined by zeta potential was found to be -31.5 and average uniformity of particles through PdI was found to be 0.207 are indicating greater stability of formulation.

TEM and SEM Analysis of optimized formulation



(a) (b)

Fig. 4 TEM (a) and SEM (b) analysis image of optimized NLC-DOPA formulation

The NLC particles were towards the spherical in shape with dark spots on the surface was shown the particle size (83.32-104.15nm) observed by TEM, which was correlated well with the outcomes obtained from particle size (85.53-106.11nm) observed by zetasizer. The surface morphology of particles by SEM analysis was found to be the spherical in shape with smooth surface.

XRD (X-Ray Diffraction)

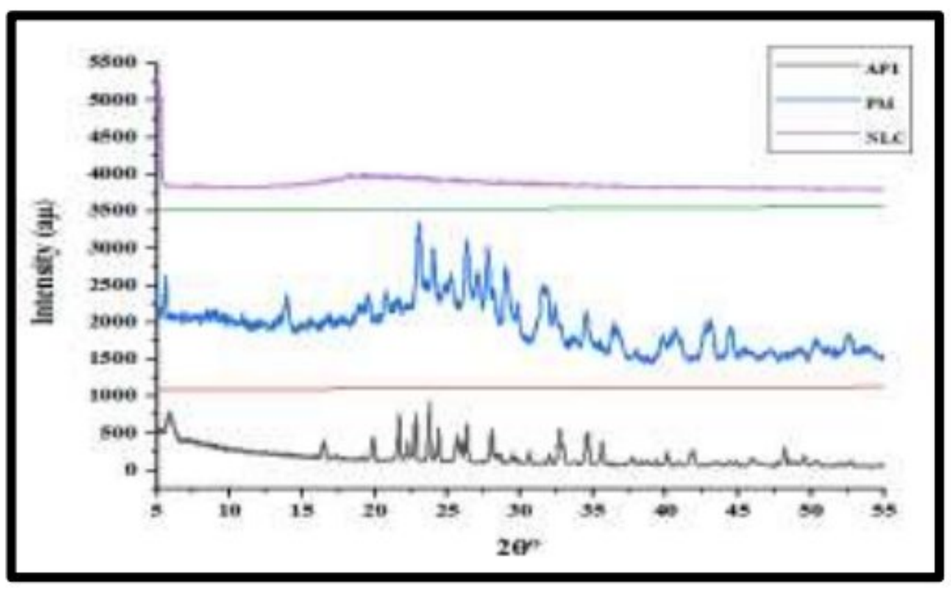
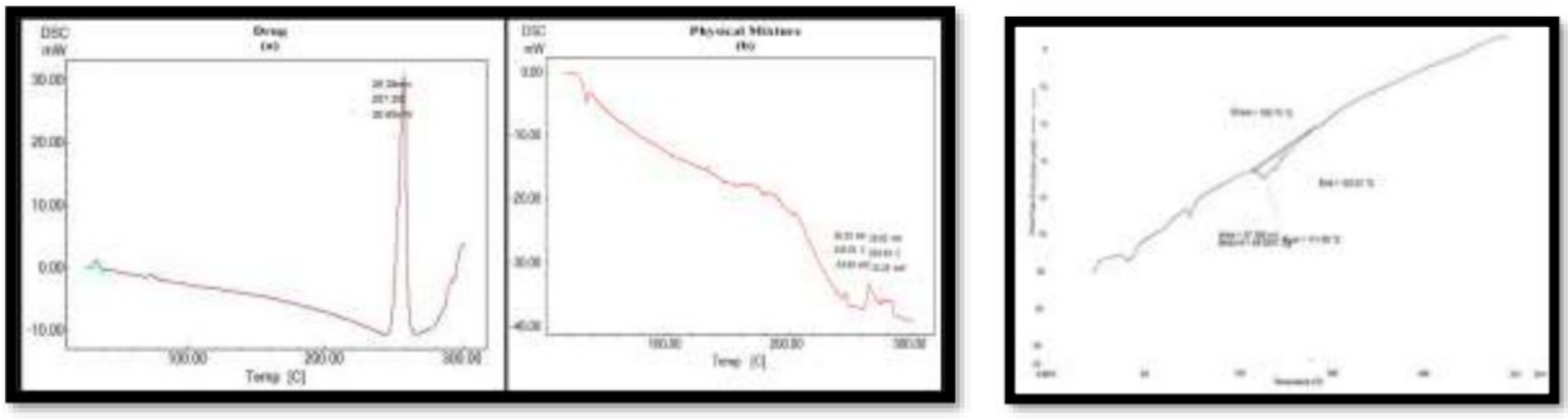


Fig. 5 XRD diffractogram of Drug, Physical mixture and optimized formulation

The X-ray diffractogram of drug showed sharp peaks (crystalline phase of drug), but these peaks vanished in the x-ray diffractogram of physical mixture and formulation, indicating that the crystalline phase of drug had changed into the amorphous phase of formulation.

DSC (Differential Scanning Colorimetry)



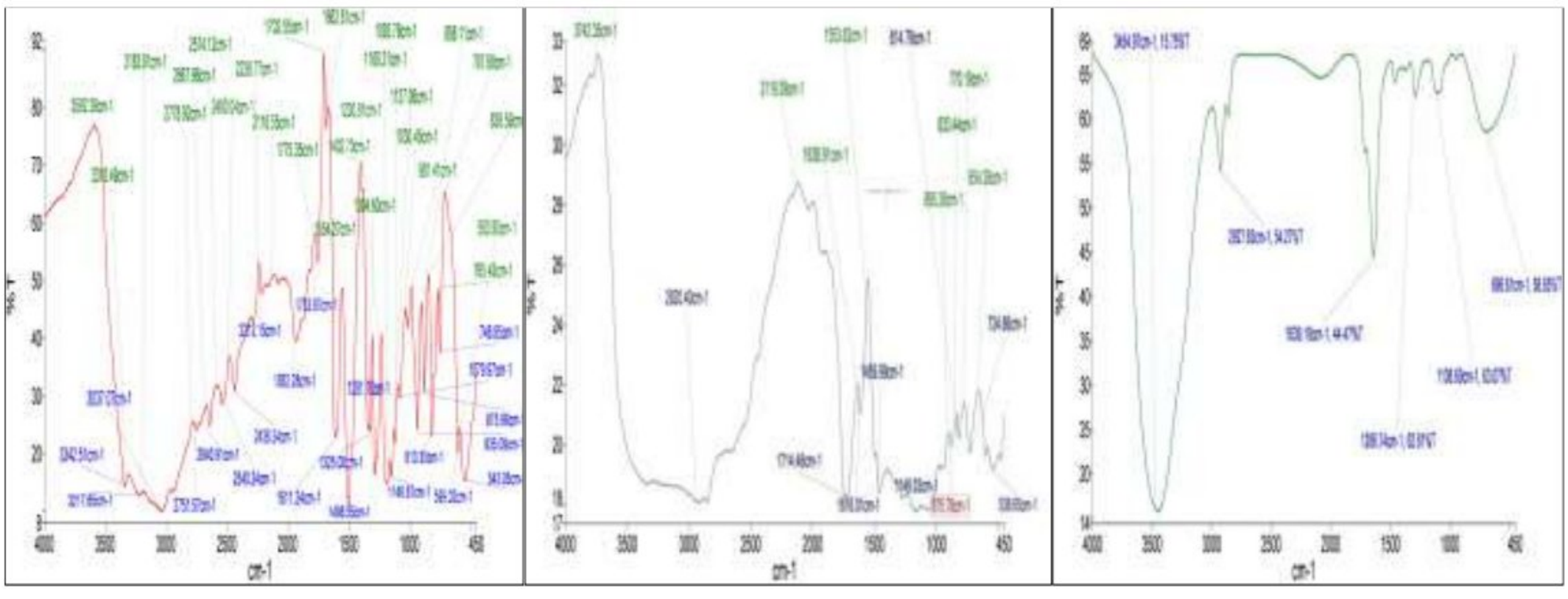
(a) **(b)** **(c)**

Fig. 6 DSC Thermo gram of Drug (a) Physical mixture of drug and excipients (b) and Optimized NLC-DOPA formulation (c)

DSC thermogram of pure drug indicating the sharp crystalline exothermic peak of dopamine hydrochloride directed upwards at 257.29^{0}C melting points.

The thermograms of physical mixture and freeze-dried drug-loaded NLC, reveal very small characteristic peaks of dopamine. Dopamine's melting point could not be detected in the thermogram of PM and NLC-DOPA formulation, which may be due to its amorphous state or molecular dispersion in the lipid matrix.

FTIR Spectroscopy Analysis



(a) **(b)** **(c)**

Fig. 7 FTIR spectrometry for Drug (a) Physical mixture of drug and excipients (b) and Optimized NLC-DOPA formulation (c)

The FTIR spectrum of drug, physical mixture and NLC-DOPA formulation were determined that the characteristic peaks of dopamine (drug) and lipids (excipients) were present in all spectra and that there was no major evolution of existing peaks or formation of new peaks. There is only a physical interaction between drugs and other substances and no chemical interactions occur.

In vivo Evaluation

Pharmacodynamic Study

Model Induced by Rotenone

Normal Control groups I = 5

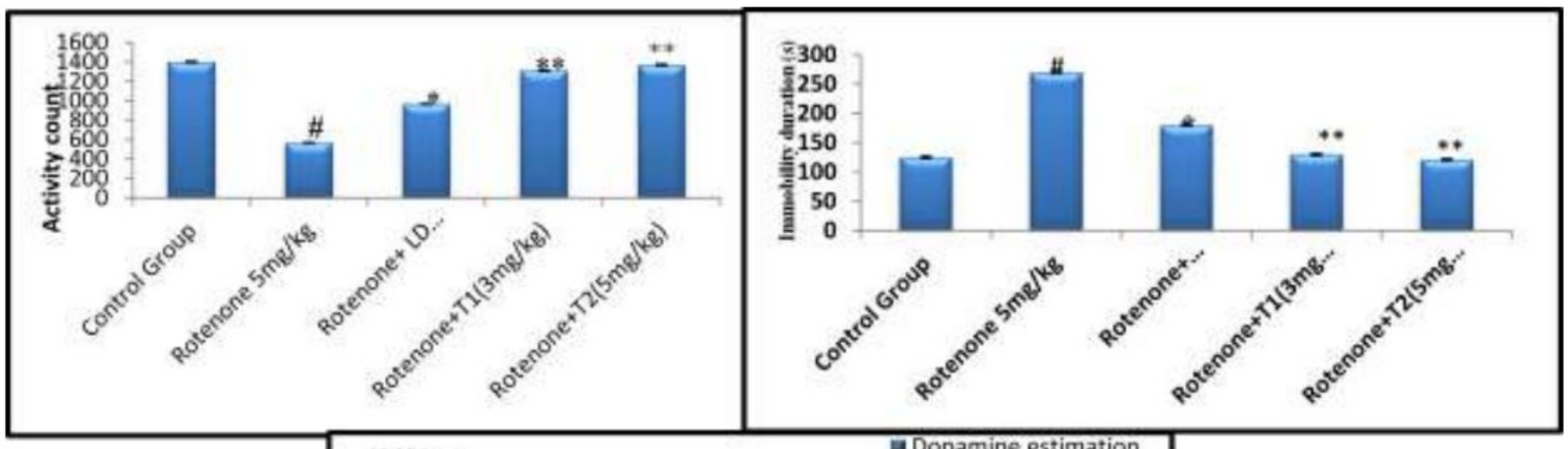
Negative control groups II (Rotenone, 5 mg/kg, orally) = 5

Positive Control III (Rotenone, 5 mg/kg, orally +Levodopa, 5mg/kg, orally) = 5

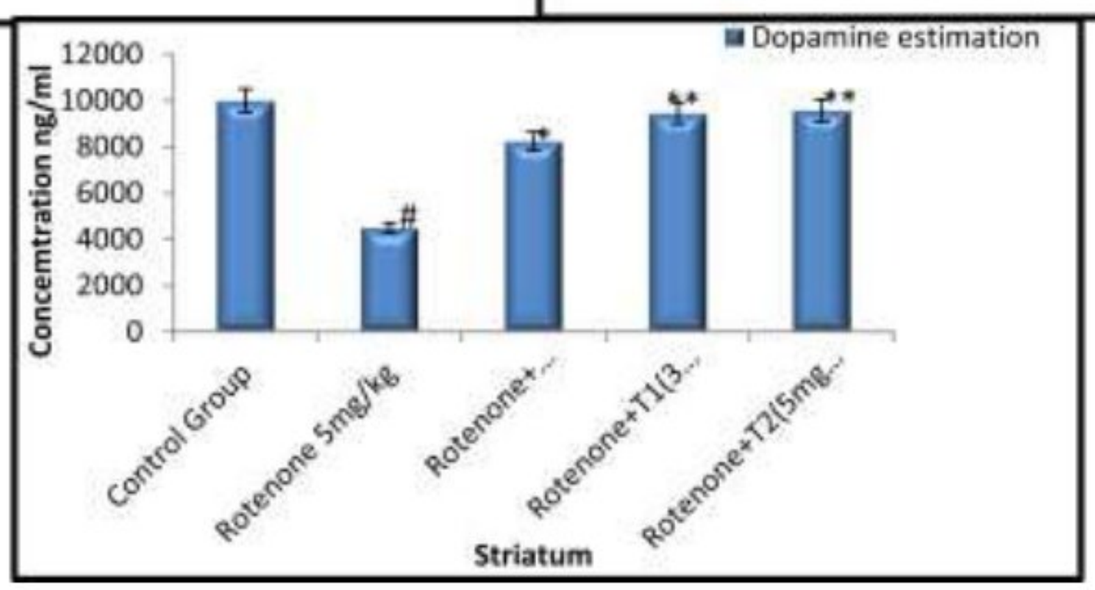
Test groups IV (Rotenone, 5 mg/kg, orally + NLC-DOPA, 3mg/kg, intranasal) = 5

Test groups V (Rotenone, 5 mg/kg, orally + NLC-DOPA, 5mg/kg, intranasal = 5

Total number of animals = 25



a b



c

Fig. 8 Locomotors activity recorded for different groups (n=5), #p<0.01; *p<0.05 vs control group (a), Force swimming test Dopamine for different groups (n=5), #p<0.01; *p<0.05 vs control group (b) and Dopamine estimation in striatum nigra (n=5), #p<0.01; *p<0.05 vs control group, no significant difference between control group and test groups (c).

Test of locomotor activity

The mean counts of rats in the rotenone-containing group II and the Levodopa-containing group III were significantly lower than in the control group I whereas there was no significant difference between NLC-DOPA-containing test groups IV and V.

Force Test of Forced Swimming

The mean value of immobility in rats in the rotenone-containing group II and Levodopa-containing group III increased significantly when compared with the control group I, but there was no difference between NLC-DOPA-containing test groups IV and V.

Neurochemical assessment

The level of dopamine was significantly decreased in rotenone containing group II and Levodopa containing group III when compared with rats in the control groups I, but there was no significant difference between NLC-DOPA containing test groups IV and V when compared with control group I.

Pharmacokinetic release in blood and brain

Table 5. Pharmacokinetic parameters in blood and drug concentration in brain

Group (n=3)	**C_{max} (ng/ml)**	**T_{max} (h)**	**$T_{1/2}$ (h)**	**AUC 0-t (ng/ml*h)**	**Vz/F_obs (mg/kg)/ (ng/ml)**	**Cl/F_obs (mg/kg)/ (ng/ml)/ h**	**Mean Concentration of drug in brain (µg/ml) ± RSD%**
API (Dopamine Hydrochloride)	360.66	0.5	3.9	913.14	0.01290	0.00230	149.36±2.86%

Marketed formulation (Levodopa)	388.85	1	3.0	933.30	0.01087	0.00249	503.87± 3.26%
Prepared Formulation (NLC-DOPA)	260.33	0.5	13.3	743.27	0.01882	0.00098	3,736.13± 1.08%

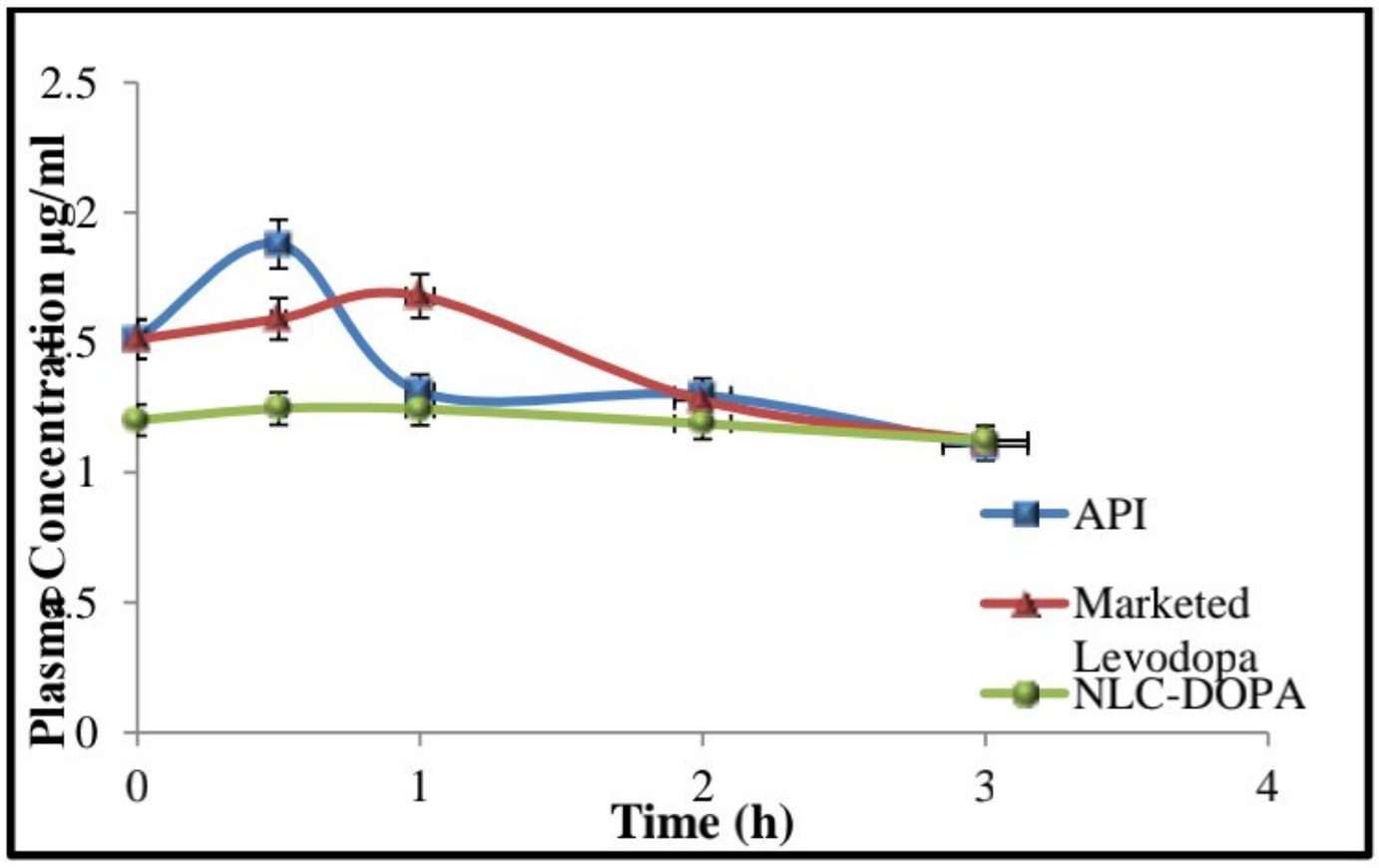


Fig. 9 Plasma concentration vs. time graph for API, marketed formulation Levodopa and prepared formulation NLC-DOPA.

The graph was indicated that the NLC- DOPA formulation produced a sustained and controlled delivery from nose to brain along with a higher significant elimination half-life than API and marketed formulation determined by PK solver 0.2 s0ftware.

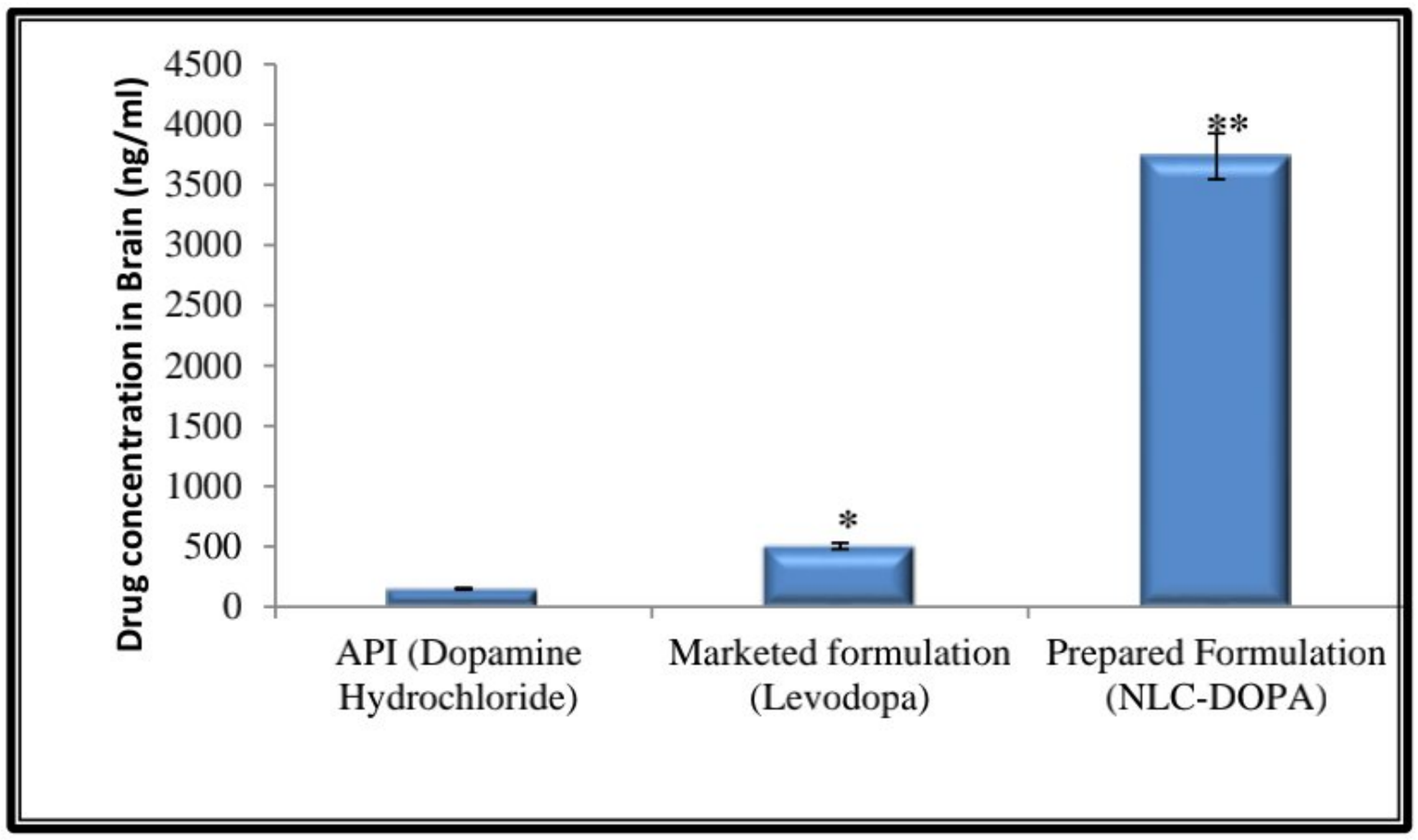


Fig. 10 Schematic graph for the drug concentration in brain, Value expressed as a mean (n=3)

*P< .05; **p< .01versus API group

The NLC-DOPA (intra nasal) was targeted to the brain, it is significantly higher than the amount of drug by API (intra nasal) and marketed Levodopa tablet (oral).

Ex vivo Study

Permeation Study

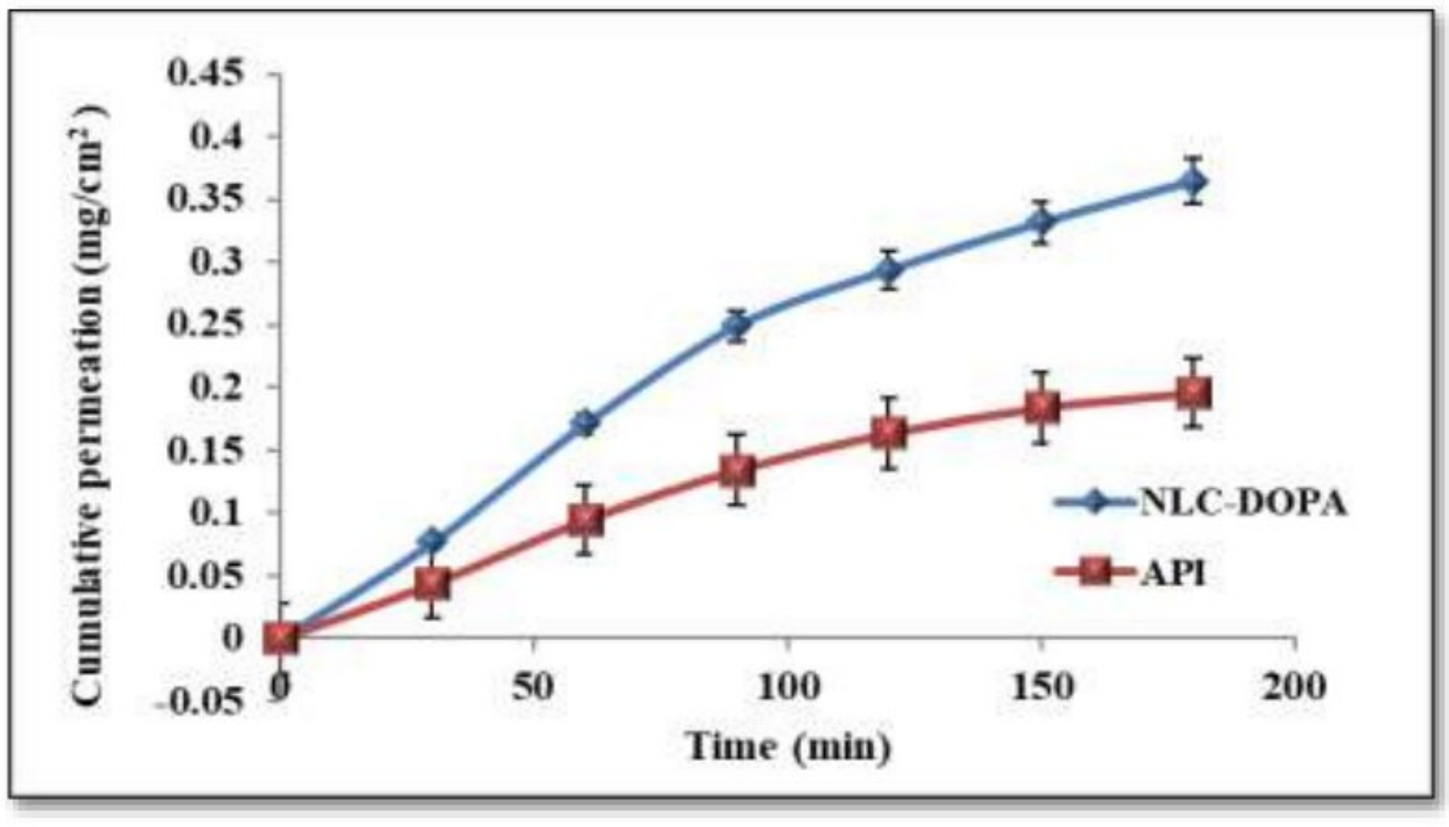


Fig. 11Permeation Study

Permeation of dopamine across the nasal mucosa was significantly higher from NLC-DOPA than from API. Approximately 36.4% of the drug permeated within 3 hours of NLC-DOPA, whereas API was shown slower permeation at the end of the same time interval. The permeability's coefficient of NLC- DOPA was found to be $6.67*10^{-4}$ cm/min, much higher than permeability's coefficient of API was found to be $3.3*10^{-4}$ cm/min.

Ciliotoxicity study

The nasal mucosa treated with saline showed no nasociliary damage and the nasal membrane remained intact, whereas an extensive damage to nasal mucosa coupled with loss of nasal cilia could be pragmatic with isopropyl alcohol. NLC-DOPA, on the other hand, did not cause any harm to the nasal mucosa, proving that the excipients used in the formulation were completely safe.

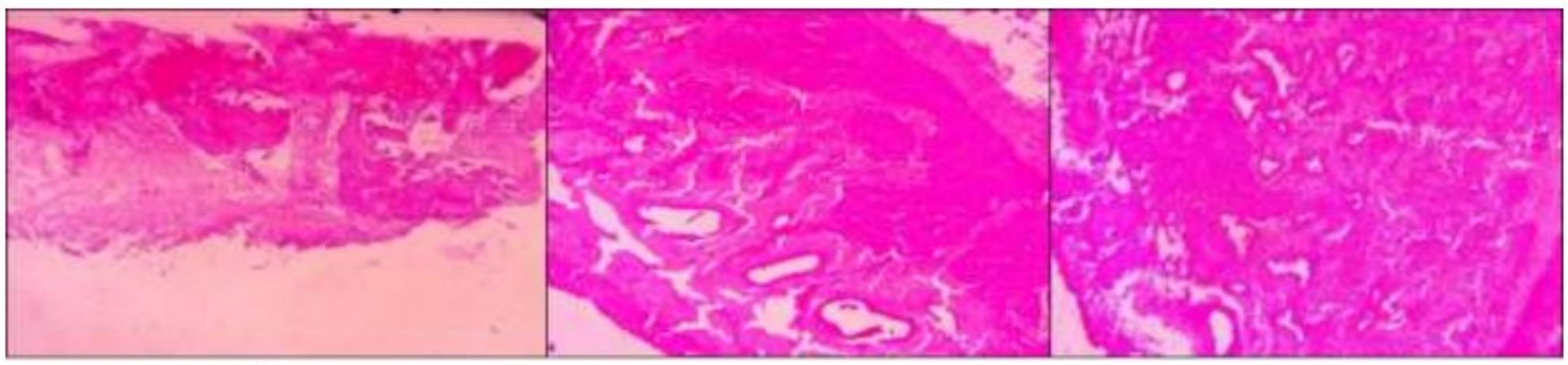


Fig.12. Histopathology images of nasal mucosa of goat treated with (A) Isopropyl alcohol (B) Saline (C) NLC-DOPA.

When the nasal mucosa was allowed to treat with saline, no nasociliary injuries occurred, and the nasal membrane survived unchanged. However, when the nasal mucosa was treated with isopropyl alcohol, extensive damage to the nasal mucosa was observed, along with the loss of nasal cilia. However, no nasal mucosa damage was seen with NLC-DOPA, suggesting that the excipients used were safe.

Stability Study

Particle size and entrapment efficiency did not change significantly when they were stored at 4 ± 2^0 C (refrigerator) and 25 ± 2^0 C / 60 ± 5% RH, but the size of particles increased due to

accumulation when they were stored at 40 ± 2^0 C / 75 ± 5% RH, while there was no change in entrapment efficiency.

Table 6. The characteristic parameters of NLC-DOPA after six months for stability studies at different condition, n=3.

Temp (^{0}C)/RH (%)	Time (months)	Characteristic parameters		
		particles size (nm)	PDI	Entrapment efficiency (%)
4 ± 2^{0}C	0	89.04±0.804	0.227±0.081	90.36±0.898
	1	90.38±0.856	0.207±0.080	90.32±0.821
	3	106.3±0.943	0.223±0.082	90.21±0.812
	6	129.7±0.860	0.234±0.082	89.86±0.898
25±2 ^{0}C/ 60 ± 5%	0	89.04±0.820	0.227±0.086	90.36±0.673
	1	90.56±0.907	0.208±0.080	89.76±0.779
	3	103.6±0.660	0.226±0.078	88.32±0.784
	6	131.7±0.920	0.236±0.078	89.64±0.816
40 ± 2 ^{0}C/75 ± 5%	0	103.06±0.808	0.226±0.073	90.34±0.735
	1	106.5±0.860	0.223±0.086	88.45±0.868
	3	129.7±0.735	0.227±0.086	88.65±0.894
	6	131.7± 0.771	0.236±0.089	89.83±0.45

Histopathology of brain

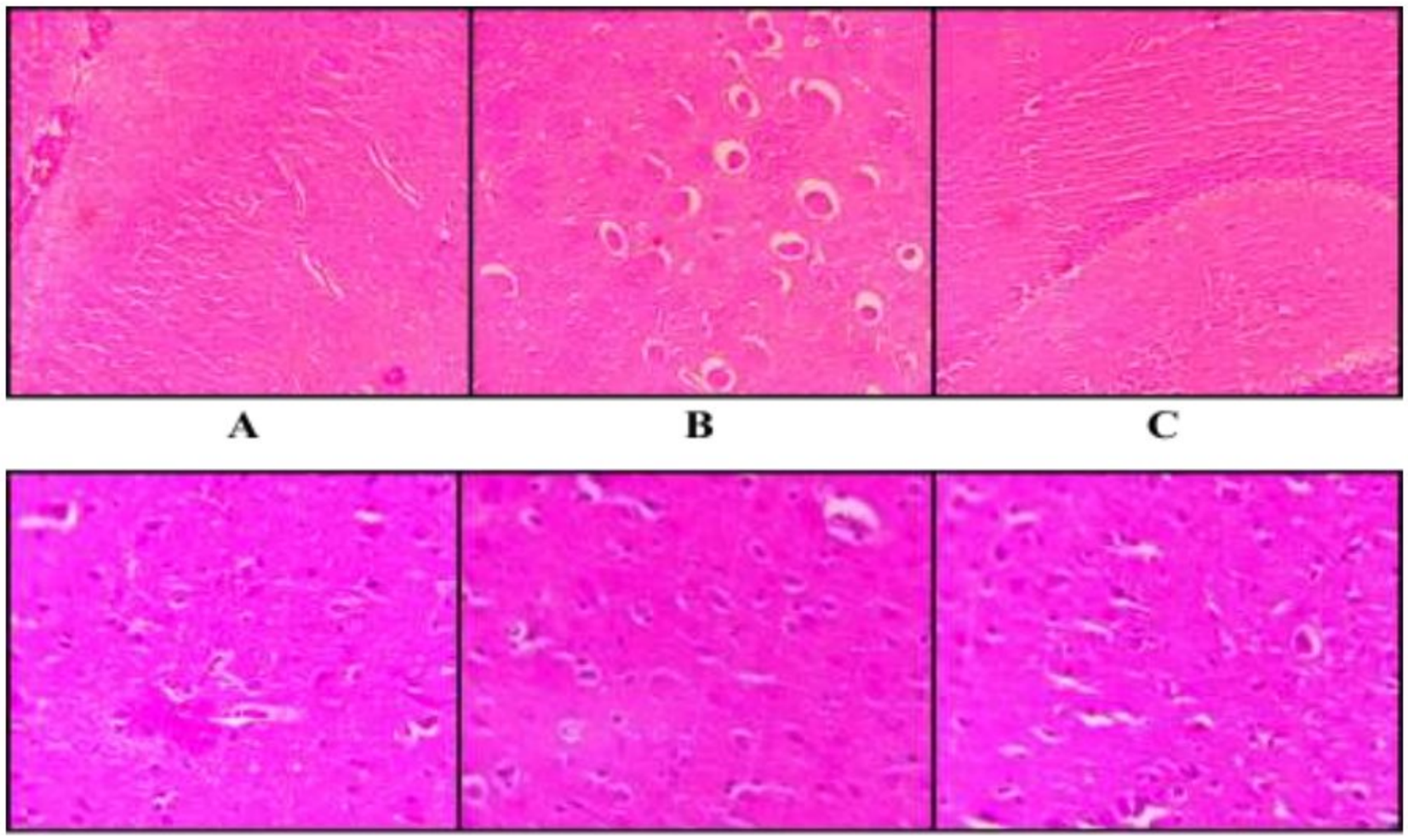


Fig.13. Histopathology of Brain cells (A-C) and striatum nigra (D-F): Control brain cells (A), Disease brain cells (B), Treated brain cells (C), Control striatum nigra (D), Disease striatum nigra (E) and Treated striatum nigra (F).

Neuron inflammatory cells and fewer neuron cells were found in disease induced striatum nigra in comparison to treated striatum nigra and control striatum nigra, whereas treated striatum nigra was more similar to control striatum nigra. Disease-induced brain cells collapsed, but treated group brain cells resembled control group brain cells more closely.

Conclusion

Dopamine HCl-loaded nanostructured lipid carriers were successfully prepared. Software optimization of formulation, based on drug content and particle morphology was successful. HPLC and UV technique validation of dopamine HCL, drug physiochemical property, and formulation stability study were determined (mentioned in 2nd and 3rd DSRC reports). All optimized formulation characterizations succeeded effectively. In vitro release kinetic was sustained and regulated. In vivo (Pharmacodynamic) investigation showed considerable formulation effects on rat model. In vivo (Pharmacokinetic) studies showed drug concentration in blood and brain and other parameters that confirmed formulation's prolonged release profile. The in-vivo study is concluded that the Intranasal delivery of NLC-DOPA effectively targeted

into brain than blood when compared to API and oral route of levodopa. The ex-vivo permeation and ciliotoxicity investigation showed no formulation toxicity during delivery and considerable penetration. Brain histology is done appropriately.

Objective Achieved

- The optimization of nanostructure lipid carrier was achieved with help of Design software expert version 13 Stat-Ease via a central composite factorial design.
- Method of validation for NLC-DOPA has been performed by UV Spectroscopy and HPLC, discussed in second and third DSRC report.
- Dopamine loaded nanostructure lipid carrier was prepared using high pressure homogenization technique.
- In vitro evaluation was performed and achieved significant results.
- In vivo evaluation was performed and achieved significant result.
- Ex vivo study was performed and achieved significant result.
- Stability study has been performed significantly and explained in third DSRC report.
- Histopathology was performed and achieved significant results.

3. Publication

Published Article

1. Neha S.L., Ashwini Kumar Mishra, Laxmi Rani, Shweta Paroha, Hitesh Kumar Dewangan, Pravat Kumar Sahoo. Design and Evaluations of a Nanostructured Lipid Carrier Loaded with Dopamine Hydrochloride for Intranasal Bypass Drug Delivery in Parkinson's disease. Journal of Microencapsulation. 2023; https://doi.org/10.1080/02652048.2023.2264386 (Accepted).

2. Neha S.L., Mishra A.K, Rani L., Verma S.P., Sahoo P.K. Characterization and HPLC method validation for determination of dopamine hydrochloride in prepared nano particles and pharmacokinetic application, Analytical chemistry letters. 2022; 12(4): 528-541. https://doi.org/10.1080/22297928.2022.2121662.

3. Neha S.L., Pannu A., Sahoo P.K. Development and validate of UV spectroscopic technique for the assessment of dopamine to prepare NLC formulation in the therapy of Parkinson's disease, Analytical chemistry letters. 2021; 11(4): 553-562. https://doi.org/10.1080/22297928.2021.1934111.

4. Neha S.L., Pannu A., Sahoo P.K. An Overview of second-generation nanoparticles; Nano stricter lipid carrier drug delivery system, International journal of pharmaceutical sciences and nanotechnology. 2020; 13(6): 5181- 89. https://doi.org/10.37285/ijpsn.2020.13.6.2.

Communication Article

1. Optimization, Formulation and Evaluation of dopamine hydrochloride loaded nanostructure lipid carrier for intranasal drug delivery in Parkinson's disease. (Research article)

Conferences

1. Oral presentation in International Conference on Sustainable materials and technologies for bio and energy applications from 13-15th, July 2022 hosted by Sri Sivasubramaniya Nadar College of Engineering, Kalavakkam, Chennai at online mode.

2. Poster presentation in international conference on recent advances in nano medical sciences hosted by Institute of nano sciences and Institution of Eminence, University of Delhi from 22 June 2022 to 23 June 2022 in Vallabhbhai Patel chest institute, University of Delhi.

P. K. Sahoo

Supervisor Name and Sign
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